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DIFFERENTIAL EXPRESSION OF CHICKEN BRAIN PROTEINS DURING DEVELOPMENT

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ABSTRACT

Understanding the proteins role and their spatiotemporal relationships in the development of brain will enrich the knowledge about structure – function relationships, control of productive traits, and neurological diseases. This study explores the different proteins involved in the development of the brain. Brains from Vanaraja breed embryos were collected from days 2 to 13continuously; from days 15,17,19,21 and from 6weeks birds and analyzed by SDS PAGE (5% stacking gel and 12% resolving gel). 27 proteins were differentially expressed in different developmental stages. Some of these proteins are 160kDa, 145kDa, 120-123kda, 100kDa, 92-102kDa, 97kDa, 80kDa, 66kDa, 60kDa, 58kDa, 50kDa, 34kDa, 22-25kDa, 20kDa, 16kDa, 14kDa. There was a progressive increase in the expression levels of higher molecular weights with advancing age. During early development of brain, may be low molecular weight proteins are playing a vital role in neural induction, anterio –posterior patterning, dorsovental patterning and cell differentiation.

***KEY WORDS** : Proteins, organogenesis, Brain, Differential expression, Gel electrophoresis*

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INTRODUCTION

Understanding the neural system and function of the brain is one of the greatest scientific challenge and unique theme of interest in general. Understanding proteins role and development and its function. The proteins which have important role in neural tube organogenesis are transcription factors like MSX1, MSX3, SOX14 AND GBX2, Clhx2[1], signaling proteins like FGF[2], WNT, BDNF, membrane protein, extracellular matrix proteins. These proteins have specific spatiotemporal distribution pattern required in development of brain. The present study was undertaken to study the differential expression of proteins during brain development. Poultry was chosen as the model animal due to ease of collection of embryos from eggs. Signaling biomolecules and signal transduction pathways are highly conserved among species and study of development of chicken brain can be used to explore mammalian brain.

MATERIALS AND METHODS

Poultry embryonated eggs of 2nd to 13 every day and 15 to 19th alternate days eggs, day old chick and 6th week adult bird of vanaraja breed were procured from project directorate on poultry, rajendranagar. Hyderabad. Brain was dissected from the above mentioned group by the method described by Freshney, 2005 in ice cold 1M TRIS Hcl buffer $_P$H- 8.3 (20Mm TRIS 4.84gm 10mm sodium azide 0.4gm, 10mM β mercaptoethanol 1.370gm, EDTA 0.6gm, PMSF 0.070gm, Tween 20 9.04 ml) to inhibit the activity of proteases and to prevent protein denaturation. The brain tissues collected from each age group were pooled, homogenization was one using hand homogenizer, centrifuged in refrigerated centrifuge (4^0C) at 11,000 rpm for 20 minutes, and supernatants were collected and preserved at -20^0 C until further analysis. The supernatants were analyzed for differential expression of proteins by SDS PAGE Bollag et al., (1996)[3] with 5% stacking gel, 12% resolving gel and Tris-glycine- SDS running buffer. Medium range molecular weight markers were used (Bangalore Genei Company; catalogue No PMWM-105979; Range- 97kDa to 14kDa).

RESULTS

The results of one dimensional SDS PAGE of brain tissue supernatants of embryos, day old chicks and 6weeks bird were analyzed by the visual examination. The protein profiles at different ages revealed some similar bands.

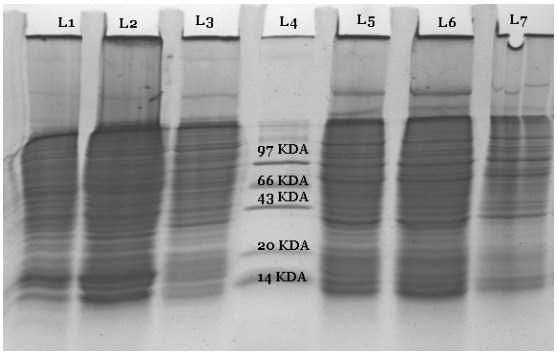

Figure- 1

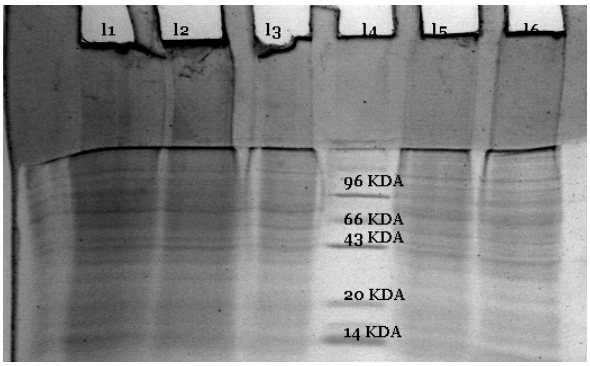

Figure- 2

Coomasie blue stained tissue extracts at different stages of brain dvelopment

L1: Day 2 embryo brain extract, L2: Day 3 embryo brain extract, L3:Day 4 embryo brain extract, L4: Molecuar weight marker, L5:Day 5 embryo brain extract, L6:Day 6 embryo brain extract, L7:Day 7 embryo brain extract, Figure 2-l1:Day 8 embryo brain extract, l2: Day 9 embryo brain extract, l3:Day 10 embryo brain extract, l4: Molecular weight marker, l5:Day 11 embryo brain extract, l6:Day 12 embryo brain extract, l7:Day 13 embryo brain extract

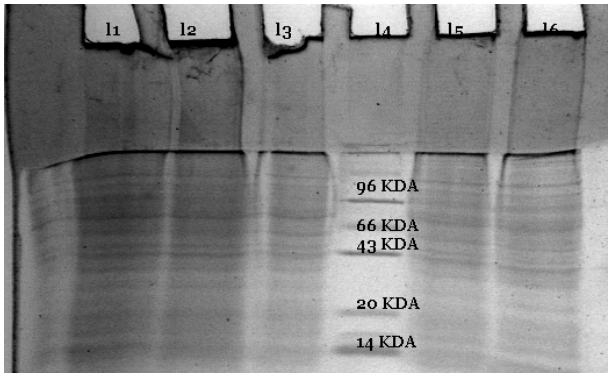

Figure -3 l1

Figure -3 l1: Day 15 embryo brain extract, l2: Day 17 embryo brain extract, l3: Day 19 embryo brain extract, l4: molecular weight marker l5: Day 1 Chick brain extract, l6: Six weeks brain extract

Table 1

Differentially expressed proteins during chicken brain development

MW	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D15	D17	D19	D21	WKS6
160	A	A	A	A	A	A	A	A	A	A	A	A	A	**	**	**	**
145	A	A	A	A	A	A	A	A	A	A	A	A	*	*	*	*	*
130	A	A	A	A	A	A	A	A	A	A	A	A	*	*	*	*	*
123	A	A	A	A	A	A	**	**	**	***	**	**	A	A	A	A	A
120	A	A	A	A	A	A	**	**	**	**	**	**	A	A	A	A	A
105	A	A	A	A	A	A	A	A	A	A	A	A	*	*	*	*	*
100	*	A	A	A	A	A	A	A	A	A	A	A	A	*	*	*	F
97	A	A	***	***	***	***	**	**	**	**	**	**	A	A	A	A	A
90	A	A	A	A	A	A	*	*	*	**	**	**	A	A	A	A	A
80	A	A	A	A	A	A	A	A	A	A	A	A	*	*	*	*	*
78	*	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

75	A	A	A	A	A	A	A	*	*	*	A	A	A	A	A	A	A
73	A	A	A	A	A	A	A	A	A	A	A	A	F	F	F	*	*
66	F	F	F	***	***	***	**	**	**	*	*	*	A	A	A	A	A
60	A	A	A	A	A	A	A	A	A	A	*	*	A	A	A	A	A
58	*	*	*	*	*	*	*	*	*	*	*	*	A	A	A	A	A
50	*	*	*	*	*	*	*	A	A	A	A	A	A	A	A	A	A
48	**	**	**	**	**	**	F	F	F	F	F	F	**	**	**	*	*
45	***	***	***	***	***	***	***	***	***	***	***	***	F	F	**	*	F
38			**	A	A	**	A	A	A	A	A	A	A	A	A	A	A
34	**	**	**	**	**	**	A	A	A	A	A	A	A	A	A	A	A
25	**	**	**	**	**	**	A	A	A	A	A	A	A	A	A	A	A
20	**	**	**	**	**	**	F	F	F	F	F	F	A	A	A	A	A
16	**	**	**	**	**	**	A	A	A	A	A	A	A	A	A	A	A
14	*	*	*	*	*	*	*	A	A	A	A	A	A	A	A	A	A
5	A	A	A	A	A	A	A	*	*	*	*	A	A	A	A	A	A
4	A	A	A	A	A	A	A	A	*	*	*	A	A	A	A	A	A

LEGEND

a.	***F***	***Faint band***
b.	*****	***prominent band***
c.	******	***very prominent band***
d.	*******	***very very prominent***
e.	***A***	***Absent***

Differentially Expressed Protein Bands depicted in Table -1 Are Described As Follows: 160 kDa: The protein band is very prominent from day 17, 19, 21 and 6wks bird.145 kDa: The protein band is prominent from day 15,17,19,21 and 6wks. 130 kDa: This band is prominent from 15th day onwards. 123kDa: It is absent from 2nd day to 7th day, which became prominent on 8th day ,9th day, 10th day increased its intensity and became very very prominent on 11th day and very prominent on 12th and 13th day. Later on from 15th day onwards it is absent. 120kDa: This protein band is absent in early days of embryonic brain development but became very prominent from 8th day to 13th day and absent from 15th day onwards. 100 kDa: The protein band is absent during initial days but prominent from 17th to 21 days and became faint on 6weeks. 97 kDa: This band is absent in 2nd day and 3rd day and became very very prominent from 4th day to 7th day. It became very prominent from 8th day to 13 day and absent from 15th day onwards.90kDa: This protein band appeared on 8th day it is prominent till 13th day and absent from 15th day onwards. 80kDa: This band is absent on all days and became prominent from 15th day.73-75kDa: These are two bands absent initially from day 2 to day 8 and appeared from 9th 10th and 11th day. 66 kDa: This band is faint in initial stages of brain development that is from 2nd day 3rd day and 4th day. It is very very prominent from 6th day, 7th day, and 8th day and very prominent from day 9th day and 10th day. From 11th, 12th, 13th days band became prominent and from 17th it is completely absent. 60 kDa: It is absent from 2nd day to 11th day. It is prominent on 12th day and 13th day. It is absent from 15th day to 6wks.58kDa: This band is prominent from 2nd day to 13th day and absent from 15th day. 50kDa: This band is prominent from 2nd day to 8th day. It is absent from 9th day onwards.48kDa: This band is very prominent

from 2nd day to7th day. Later on it became faint from 8th day to 13th day and became very prominent from 15th day to 19th day. On 21st day and 6wks it became faint.45kDa: This is interesting protein band which is very very prominent in all the days of embryo except on 15th and 17th where it is faint became very prominent on 19th day and prominent on 21st day and became faint in 6wks.38kDa:It is very prominent on 2nd day, 3rd day, 4th day, and absent in 5th , 6th day and very prominent on 7th day and absent from 8th day onwards.34kDa: It is very prominent from 2nd day to 7th day and is absent from day 8th day onwards.25kDa: It is very prominent from 2nd day to 7th day and is absent from 8th day onwards. 20kDa: This protein band is prominent in from 2nd day to 7th day and became faint from 8th day to 13th day and absent from 15th day onwards. 16kDa: This band prominent from 2nd day to 7th day and absent from 8th day onwards. 14kDa: This protein band is very prominent from 2nd day to 6th day. On 7th day it became prominent and it is fainted on 9th day and absent from 10th day onwards.5kDa: Absent initially till 8th day. Later from 9th day it is prominent till 12th day and absent from 13th day onwards. 4kDa, It is absent on all the days except for it is prominent on 10th, 11th, 12th days.

DISCUSSION

160 kDa: This protein could be kinectin an integral membrane protein[4], playing an important role in development of cerebellum. 145 KDa: it could be Aggrecan, present as part of extracellular matrix and plays important roles in morphogenesis by modulating cell matrix or cell- cell interactions, cell adhesions or in binding and presenting growth and differentiation factors. Its expression is prominent in chick brain from 15th day, to 6wks. However earlier studies found that aggrecan is expressed from 7th day[5]. It regulates astrocyte development, and astrocyte differentiation. Aggrecan may be playing role in control of giant cell maturation during brain development[6]. 130 kDA: The expression of 130 kDa PLC-β4 in cerebellum was gradually increased from 5 days after birth to adulthood. In the adult cerebellum, especially high level of 130 kDa PLC-β4 was localized on the periphery of Purkinje cells in the patch-like pattern. This implies that 130 kDa PLC-β4 may a role in cerebellar Purkinje cells. 120-123 KDa: These may be APPs, transmembrane proteins expressed in several cell types. In nervous system, APP is expressed by glial and neuronal cells. It is involved in interactions with extracellular matrix components, including laminin. It plays pivotal role in morphological differentiation of cortical neurons in primary culture[7]. Proliferation of cortical cells start on E18 and E19 and maximum proliferation is observed on E19 and E20. APP could be playing an important role in cortical development[8]. 92-102 KDa: Development of telencephalon involves the coordinated growth of diversely patterned brain structure. These could be β catenins playing an important role in dorso ventral patterning, broader effects on proliferation form day 11 to 15 and neural crest development[9,10]. 90 KDa: This protein could be Amyloid precursor protein which is required for neurogenesis [11]. 75 KDa: it is significantly present in temporal, occipital and caudate nucleus and combats neuronal cell death[12]. This protein could also be a hyaluron(H_A) receptor for RHAMM (receptor for HA mediated motility), has been shown to play a critical role in mechanisms underlying the motile capacity of a variety of peripheral cell types and it is involved in mediation of neurite motility and migration. 66 KDa: This could be GPI brevican[13]. It is a member of the aggrecan/versican family of proteoglycans, anchored to the cell surface via a glycophosphatidyl ionositol (GPI) moiety. GPI linked brevican is predominantly expressed in glial cells and its tight association with brain myelin fractions suggests a role in neuroglia[14]. 60 kDa: It could be occludin, a transmembrane protein causes electrical resistance in blood brain barrier[15]. Besides this it can also be a

calcium binding protein which regulates calcium levels in brain[16]. Studies conducted in amphibians and bovines show that this protein is playing role in calcium regulation. 48KDa: This protein could be important in Synapse formation, stratification of Cerebellar cortex, development of purkinge cells in cerebellum[17]. 45KDa: This protein is present in grey matter. It is always present in combination with 20 kDa which is absent in multiple sclerosis victims. This protein could be playing a role in myelin formation. 38KDa: This protein constitutes an important mode of synaptic inhibition in nervous system.22-25 KDa: This protein could be claudin. Much of the cell membrane and cell structure is formed during early stages of embryo development. Claudins take part in development of membrane structures. . 20 kDa: Could be shhN protein present in grey matter normally. This is absent in patient's suffering with multiple sclerosis; perhaps it is playing an important role in myelination[18]. 16 KDa: FGF Development of brain and pattering of nervous system requires fibroblast growth factors much of the development of brain takes place from day 2 to day7. In the gel analysis FGF appeared from 2nd day to 7th day and faints form day 8. 14KDa: This protein band could be myelin basic protein[19]. It is important protein in myelination.

CONCLUSION

There was a progressive increase in the expression levels of higher molecular weights from 15th day. During early development of brain low molecular weight proteins are playing a vital role in neural induction, anterio – posterior patterning, dorsovental patterning and cell differentiation. Based on earlier work architecture of brain is formed from 1st day to 12th day, increased myelination is observed from 13th day to 21st day. After hatching different membrane proteins are involved in cognitive abilities of chick.

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